Agent for the photodynamic diagnosis and therapy of malignant tumours

Description

The invention concerns an agent for the photodynamic diagnosis and therapy of oncological diseases based on chlorin E_6 compounds and new medical uses of chlorin E_6 compounds.

An agent is described in the Russian patent No. 2152790 for the photodynamic diagnosis and therapy of oncological diseases which is composed of 40 to 90 % by weight chlorin E_6 and 60 to 10 % by weight polyvinylpyrrolidone.

Photosensitizers are necessary for photodynamic cancer therapy. These sensitizers are injected and accumulate mainly in the cells affected by the cancer. The production of cytostatic substances is induced in the cancer cells which contain the photosensitizer by the targeted action of laser light of a certain wavelength. This results in a tumour necrosis.

Known sensitizers for this purpose are haematoporphyrins, phthalocyanines and naphthalocyanines. In practice sensitizers based on porphyrin have proven successful due to their low phototoxicity and suitable sensitivity to the lasers that come into consideration for use.

The combination preparation defined above consisting of a complex of chlorin E_6 and polyvinylpyrrolidone (PVP) has proven to be particularly suitable. Chlorin E_6 has intensive adsorption bands in the spectral range of 660 ± 10 nm which is of particular importance for photodynamic therapy. However, the extinction coefficient for haematoporphyrins is relatively low. Hence a higher concentration of this sensitizer has to be introduced into the cancer cell for a successful photodynamic

therapy. Since some of the porphyrins are phototoxic, the advantage of chlorin E_6 is that it is rapidly eliminated after administration. Only 4 to 6 % of the administered amount is still detectable in the human body 24 hours after administration.

However, chlorin E_6 and its salts are relatively unstable in solution as well as in a lyophilized state at room temperature.

The complex described above of chlorin E_6 with polyvinylpyrrolidone in the stated composition has a considerably improved stability and therefore allows a considerably better application in practice. However, its accumulation in cancerous tissue compared to healthy tissue is still not quite satisfactory.

Hence the object of the invention is to eliminate the above disadvantages and to provide an agent based on chlorin E_6 for photodynamic therapy which not only has a stability that is well suited for handling but also has a substantially improved accumulation factor in cancerous tissue compared to healthy tissue. Another object of the invention is to provide further medical uses of chlorin E_6.

This object is achieved according to the invention by an agent for photodynamic therapy based on chlorin E_6 and its derivatives, and polyvinylpyrrolidone, which is characterized in that it contains or is composed of chlorin E_6 and polyvinyl-pyrrolidone in a weight ratio of 1: (> 1.5). A preferred weight ratio of chlorin E_6 to polyvinylpyrrolidone is 1: (> 1.5 to 25), preferably 1:2, more preferably 1:3, more preferably 1:5, even more preferably 1:10, even more preferably 1:15 and even more preferably 1:25.

Hence the weight ratio is preferably in the range of about 1: (5 to 25), preferably 1: (10 to 25), more preferably 1: (15 to 25), and more preferably 1: (15 to 20). The chlorin E_6 and the polyvinylpyrrolidone are preferably present as a complex.

Surprisingly, an agent having the composition stated above enables of chlorin E_6 to be accumulated in cancerous tissue compared to healthy tissue with a several-fold higher selectivity. This substantially increased accumulation not only allows the use of a lower dose of the agent but also increases the effectiveness of the laser treatment at greater depths which can be increased from previously about 1 cm penetration depth through the skin into the tissue to twice this depth.

In order to prepare the agent according to the invention, polyvinylpyrrolidone is appropriately dissolved in an aqueous base suitable for injection and then chlorin E_6 is added while continuously stirring in the amount required to achieve the desired composition of more than 1.5 and up to 25 parts by weight PVP to 1 part by weight chlorin E_6 and stirred until a completely homogenous mixture has formed. The solution obtained can be sterilized by filtration and can be freeze-dried and stored in this form at normal temperature. The formulation can also be prepared in such a manner that it is suitable for a systemic and/or local action by parenteral, enteral and/or topical administration.

The superior effectiveness of the agent according to the invention is surprising since the above-mentioned Russian patent expressly cautions against using more than 60 % by weight polyvinylpyrrolidone since excess amounts no longer react with chlorin E_6 and represent unnecessary ballast. This prejudice is overcome by the invention and a several-fold higher accumulation is achieved without reducing the stability of the complex.

A preparation of 6 to 12 kDa is preferred as the polyvinylpyrrolidone.

A complete tumour necrosis up to a depth of 20 mm was observed in animal experiments at a dose of the agent according to the invention of 1 to 5 mg/kg and laser irradiation at a wavelength of 660 nm and an energy exposition of 50 J/cm^2. Under otherwise identical conditions necroses were observed with the known

complex only up to a depth of 16 mm, a corresponding dose of chlorin E_6 alone only resulted in a partial tumour necrosis of up to 7 mm depth. The toxicity measured as the LD50 was determined to be less than 140 mg/kg. Chlorin E_6 is appropriately used in the form of its alkali salt. Derivatives of chlorin E_6 (13-carboxy-17-[2-carboxyethyl]-15-carboxymethyl-17,18-transdihydro-3-vinyl-8-ethyl-2,7,12,18-tetramethylporphin) such as the corresponding 15-carboxyethoxymethyl or 15-formyl compounds which all occur naturally as accompanying substances of chlorin E_6 are suitable in the same way. In particular the complex according to the invention can also contain mixtures of chlorin E_6 with its derivatives.

The agent according to the invention is usually administered in the form of an injectable solution. However, it is also possible to incorporate it into ointments or liniments for direct application on the skin. An amount of 0.5 to 10 mg/kg, preferably 1 to 7 mg/kg is recommended as the dosage.

It is also possible to prepare the agent according to the invention in liquid or semi-solid pharmaceutical formulations. Formulations for topical, intravenous and/or systemic administration and in particular for a systemic and/or local action are particularly preferred.

In connection with the present invention it was also found that chlorin E_6 is suitable for preparing pharmaceutical preparations for other applications than for tumour diseases.

Surprisingly, it turned out that chlorin E_6 is very effective on the skin so that skin diseases can be readily treated with agents containing chlorin E_6 and PVP as preventive measures and also for treatment. In particular, chlorin E_6-PVP is effective against fungal diseases as well as psoriasis and similar skin diseases. It is effective against dermatophytes, moulds and yeasts.

Furthermore it has also turned out that chlorin E_6 is surprisingly suitable for epilation i.e. for hair removal.

Hence another subject matter of the present invention is an agent for the prophylactic or therapeutic or cosmetic treatment of the skin, especially for treating fungal diseases of the skin, psoriasis or for hair removal, wherein the agent comprises chlorin E_6 and PVP in any mixing ratio. The weight ratios of the two components can comprise an excess of chlorin E_6 or also an excess of PVP. In particular weight ratios of chlorin E_6 to PVP of about 1 : 0.1 up to a considerable excess of polyvinylpyrrolidone compared to chlorin E_6 and in particular up to 1 : 25 are suitable. Weight ratios of 1 : 1 are particularly preferred. However, ratios of 1 : ($\geq$ 1.5), 1 : 5 or 1 : 10 or 1 : 15 are also suitable for the said applications.

The following examples illustrate the effectiveness of the agent according to the invention compared to a known agent according to the Russian Patent No. 2152790 with regard to tumour effectiveness and the effectiveness of chlorin E_6 for treating the skin.

Example 1

An agent referred to as Fotolon according to the Russian Patent No. 2152790 having the composition chlorin E_6 : polyvinylpyrrolidone in a weight ratio of 1 : 1 and an agent according to the invention having the composition chlorin E_6 : polyvinyl-pyrrolidone in a weight ratio of 1 : 10 were examined.

The investigations were carried out on 12 white raceless rats weighing between 150 and 180 g with an intraabdominally transplanted Pliss lymphosarcoma. On the 5th day after tumour transplantation all 4 groups of animals (3 rats in each group per preparation) were intravenously administered Fotolon or the agent according to the invention in a dose of 5.0 mg/kg body weight.

The accumulation dynamics of Fotolon or the agent according to the invention were observed in the tumour tissues of the rats (Pliss lymphosarcoma) and the healthy tissues (in the skin on the opposite side of the thigh) with the aid of computer-controlled fluorescence spectrophotometry using the analyser "LESA-6" (diagnostic laser "LGH 633-25" (figure 1)).

The measurements were carried out each hour during the 8 hours after administration of the preparations and after 24 hours.

The individual and average accumulation data of the preparation in the 12 rats are shown in tables 1 to 4.

Table 1: Accumulation dynamics of Fotolon in healthy tissues of rats with Pliss lymphosarcoma

Time	1h	2h	3h	4h	5h	6h	7h	8h	24h
No. 1	372	913	1067	996	889	823	829	760	495
	423	839	929	992	880	817	858	758	473
	380	806	852	939	907	835	855	766	511
	391	902	936	1004	898	829	832	773	507
	536	897	934	965	876	836	806	797	489
No. 2	411	526	699	662	663	616	637	563	512
	380	588	712	628	685	683	631	608	587
	372	620	734	770	714	658	659	671	560
	498	576	714	691	699	689	661	594	545
	546	639	687	743	684	611	621	562	590
No. 3	506	782	811	843	775	755	709	712	534
	428	689	756	767	680	631	642	584	525
	398	652	721	735	669	667	653	611	536
	411	793	811	892	721	688	696	646	587
	402	746	809	857	704	691	678	623	544
x±	430.3	731.2	811.5	832.3	762.9	721.8	717.8	668.5	533.1
Sx	15.6	33.03	28.9	33.2	24.9	30.0	23.3	21.8	9.5

Table 2: Accumulation dynamics of Fotolon in tumour tissue of rats with Pliss lymphosarcoma

Time	1h	2h	3h	4h	5h	6h	7h	8h	24h
No. 1	1428	2422	3690	4030	3646	3178	2470	2255	1465
	1318	2359	3448	4209	3212	3017	2375	2214	1403
	1351	2218	3528	4028	3106	2990	2242	2205	1492
	1557	2330	3466	4250	3624	3105	2386	2101	1468
	1458	2221	3322	4194	3514	2998	2237	2138	1489
No. 2	1066	1561	2533	2895	3324	2810	2213	2111	1564
	1077	1694	2538	2951	3291	2888	2115	2016	1684
	1044	1850	2771	3083	3278	2967	2253	2117	1614
	1121	1993	2649	2825	3127	2930	2178	2005	1588
	1096	1843	2651	3136	3184	2803	2169	2104	1560
No. 3	1223	2113	3246	3784	3113	2835	2340	2111	1623
	1325	2235	3126	3630	3087	2769	2254	2147	1648
	1267	2156	3090	3475	2970	2834	2365	2138	1705
	1284	2173	3117	3657	2785	2812	2411	2119	1655
	1311	2328	3215	3712	3116	2809	2389	2108	1589
x±	1261.7	2099.7	3092.7	3590.6	3225.1	2916.3	2293.1	2125.9	1569.8
Sx	40.2	66.4	98.1	130.6	60.3	31.7	26.9	16.9	23.1

Table 3: Accumulation dynamics of the agent according to the invention in healthy tissues of rats with Pliss lymphosarcoma

Time	1h	2h	3h	4h	5h	6h	7h	8h	24h
No. 1	666	744	988	1105	1120	1141	1045	911	712
	673	735	963	1138	1163	1108	997	932	795
	636	721	1012	1120	1102	1128	1102	914	773
	612	691	996	1163	1109	1120	1008	930	689
	644	696	925	1132	1195	1137	989	911	704
No. 2	788	813	1131	1231	1247	1174	1115	1076	811
	749	829	1115	1215	1275	1182	1095	938	773
	805	825	1109	1209	1252	1246	1117	990	735
	731	818	1098	1298	1307	1214	1003	895	791
	707	793	1150	1250	1258	1185	990	978	806
No. 3	698	788	946	1121	1137	1210	1118	1026	921
	712	813	938	1132	1182	1289	1045	966	885
	722	768	980	1098	1148	1303	1132	992	830
	741	824	1034	1117	1163	1241	1067	969	813
	708	775	1057	1134	1190	1266	1054	973	867
x±	706.1	775.5	1029.5	1164.2	1189.9	1196.3	1058.5	960.1	793.7
Sx	13.9	12.3	19.5	15.7	16.6	16.3	13.5	12.7	17.2

Table 4: Accumulation dynamics of the agent according to the invention in tumour tissues of rats with Pliss lymphosarcoma

Time	1h	2h	3h	4h	5h	6h	7h	8h	24h
No. 1	2453	3776	6898	7683	8934	9115	7770	6235	3501
	2378	3930	6979	7326	8930	9087	6823	5167	3639
	2346	3979	6815	7435	8805	8992	7590	5280	3603
	2482	3831	6723	7762	8980	9046	6805	5330	3654
	2398	3678	6516	7996	8902	9087	7672	4289	3625
No. 2	2662	3890	6930	8096	9112	9207	7012	5654	3701
	2696	4012	6914	8135	9289	9246	7046	5720	3790
	2524	3965	6918	8248	9293	9301	7994	5612	3794
	2483	3894	6896	8113	9307	9412	7023	5750	3845
	2708	3979	6727	8260	9315	9385	7110	5711	3810
No. 3	2776	4112	7313	9080	9224	9305	7087	4693	4025
	2730	4102	7222	9217	9336	9378	7116	5166	3986
	2560	4137	7290	9112	9217	9402	7023	5668	3912
	2684	3980	7572	9304	9341	9235	7114	5713	4048
	2712	4047	7284	9132	9304	9198	7200	4690	4115
x±	2572.8	3954.1	6999.8	8326.6	9151.9	9226.4	7225.7	5373.7	3802.5
Sx	37.3	32.8	72.4	174.1	49.1	35.6	91.9	132.3	47.8

Analysis of the results obtained confirms that a substantially improved selective storage in tumour tissue of rats is observed with the agent according to the invention – see figure 1 and 2 of the drawings.

Table 5: Coefficient of the accumulation selectivity of Fotolon

Time	1h	2h	3h	4h	5h	6h	7h	8h	24h
tumour	1261.7	2099.7	3092.7	3590.6	3225.1	2916.3	2293.1	2125.9	1569.8
thigh skin	430.3	731.2	811.5	832.3	762.9	721.9	717.8	668.5	533.1
coefficient	2.93	2.87	3.81	4.31	4.23	4.04	3.19	3.18	2.94

Table 6: Coefficient of the accumulation selectivity of the agent according to the invention

Time	1h	2h	3h	4h	5h	6h	7h	8h	24h
tumour	2572.8	3954.1	6998.8	8326.6	9151.9	9226.4	7225.7	5373.7	3802.5
thigh skin	706.1	775.5	1029.5	1164.2	1189.9	1196.3	1058.5	960.1	793.7
coefficient	3.64	5.23	6.80	7.15	7.69	7.71	6.83	5.60	4.79

Example 2

Tumour investigations

-preclinical investigations of Fotolon (chlorin E_6 – PVP 1:10) with regard to phototoxicity in a HET-CAM assay

The HET-CAM (chorioallantoic membrane) bioassay is important for evaluating the effects of photodynamic therapy on vessels and transplanted tumours. An advantage of the assay over animal models is that morphological changes in vessels and changes in blood perfusion/circulation can be qualitatively and quantitatively determined in vivo in a non-invasive manner. There are numerous experimental approaches to investigating the effect of phototoxic substances on incubated hen's eggs with regard to the site of application, the time of application and the criteria for assessing the findings and evaluation.

The CAM as part of the extraembryonic vascular system was selected as the site of application. The CAM is highly vascularized, transparent and develops very dynamically between day 3 and 12.

In the first investigations a comparison was carried out between Photosan-3 (haematoporphyrin derivative), Seehofer Laboratorium GmbH Company and Fotolon (chlorin E_6 – PVP 1:10) with regard to toxicity without irradiation, variable light dosage and variable photosensitizer concentration.

The following results have so far been found:

- in the case of Fotolon no toxicity occurs without irradiation
- a dose-dependent phototoxic reaction was found as a function of the Fotolon concentration and the light dose at the same power density.

Example 3

Investigations on extending the applications

In order to determine the improved efficacy of Fotolon (as a combination of chlorin E_6 and PVP in various compositions including 1:1, 1:10) compared to pure chlorin E_6, investigations were firstly carried out with pure chlorin E_6 without adding PVP. These included:

1. the treatment of psoriasis
2. the treatment of human fungal diseases in vitro and in vivo (athlete's foot) and
3. the epilation of hairs.

Treatment of psoriasis:
Chlorin E_6 was applied topically in a gel on the corresponding skin areas of a patient and allowed to act for 30 min under occlusion. Irradiation was carried out with a laser at a wavelength of 662 nm for 3 to 5 minutes and a dose of 36 to 60 J/cm^2. A UVB irradiation (without further addition of a medicinal drug) of psoriasis-damaged skin areas was carried out as a comparison.

An initial result was that the skin areas treated with PDT healed more rapidly.

The investigations on the treatment of human fungal diseases were carried out in the following simplified manner:
A small amount of the fungal tissue is removed from patients with a fungal disease for a pathological determination. A portion of this is transferred to a nutrient medium. The resulting colonies are separated and separately subjected to a photodynamic therapy (PDT).

In order to prepare for the PDT the individual fungal colonies were overlayered with a photosensitizer solution. The concentration of the photosensitizer in the solution was varied (among others 5, 10, 20 % and without a photosensitizer solution as a control) in order to determine the concentration range in which the photosensitizer acts. After the photosensitizer solution had acted on the fungal cultures for 30 minutes, they were irradiated. Among others it was examined how often the irradiation has to be repeated and at which intensity in order to achieve an adequate effectiveness.

Up to now the following fungal species have been included in the PDT with chlorin E_6:

Moulds: **Aspergillus niger, Aspergillus fumigatus, Penicillium species, Mucor species**
Yeasts: **Candida albicans, Candida glabrata**
Dermatophytes: **Trichophyton rubrum, Microsporum gypseum, Trichophyton mentagrophytes**

The results can be interpreted as follows:

Photodynamic therapy has the largest effect in the treatment of dermatophytes. The best results were obtained with the fungal cultures **Trichophyton mentagrophytes** and **Trichophyton rubrum**. In contrast PDT had no significant effect on the fungal culture **Microsporum gypseum**.

Among the various moulds a visible effect of photodynamic therapy is observed on the fungal cultures **Penicillium species**, **Aspergillus fumigatus** and **Aspergillus niger**. In contrast the fungal **species Mucor** was sensitive to PDT.

Different results were also obtained for yeasts. PDT treatment had a major effect on **Candida albicans** (the irradiated colonies died off completely). However, the fungal culture **Candida glabrata** was insensitive to PDT until the end of treatment.

Treatment of a patient with athlete's foot (**Aspergillus fumigatus**) by chlorin E_6 and subsequent irradiation resulted in a regression of the fungus. Chlorin E_6 was incorporated into a commercial gel and applied topically. Other parameters of the treatment were: chlorin E_6 concentration = 0.2 %, a laser having an irradiation wavelength of 662 nm and a power of 200 mW was used as a light source, the number of irradiations was 6, the irradiation was repeated weekly, irradiation time: 3 minutes for the first two irradiations and 5 minutes for the subsequent four irradiations.

However, treatment of one patient does not provide reliable information on the actual effectiveness of chlorin E_6. A larger number of patients were included in the treatment with Fotolon in various compositions (1:1, 1:10).

Hair epilation:

Hair epilation was carried out on various hair areas of the patient. Chlorin E_6 was incorporated into a commercial gel to a final concentration of 0.1 % and 0.2 % and applied to the corresponding skin regions. After 30 minutes time to take effect the hairy surface was irradiated for 3 to 5 minutes with a laser at an energy dose of 36 J/cm^2 or 60 J/cm^2. The hairs were subsequently removed (razor, tweezers). The chest, groin, upper lip and lower abdomen were selected as the hair areas. Depending on the success of the treatment, the irradiation was repeated once weekly within 2 to 4 weeks.

The following results were found:

- chest: most hairs did not grow again, the hairs which did grow again are very fine
- groin: most hairs did not grow again, the hairs which did grow again are very fine
- upper lip: hairs did not grow again
- lower abdomen: hairs did not grow again

These results show the effectiveness of chlorin E_6 in the treatment of psoriasis, individual fungal diseases and epilation.

Claims

1. Agent for the photodynamic diagnosis and therapy of tumours based on porphyrin compounds and polyvinylpyrrolidone,
wherein
the proportion of chlorin E_6 or a derivative thereof relative to polyvinylpyrrolidone is 1 : (> 1.5).

2. Agent as claimed in claim 1,
wherein
the weight ratio of chlorin E_6 or a derivative thereof to polyvinylpyrrolidone is 1: (5 to 25).

3. Agent as claimed in claim 1 or 2,
wherein
the weight ratio of chlorin E_6 or a derivative thereof to polyvinylpyrrolidone is 1 : (15 to 25).

4. Agent as claimed in one of the claims 1 to 3,
wherein
the polyvinylpyrrolidone is 6 to 12 kDa.

5. Use of chlorin E_6 and polyvinylpyrrolidone to produce an agent for the therapy and/or prophylactic and/or cosmetic treatment of skin.

6. Use as claimed in claim 5 for the therapy or prophylactic treatment of fungal diseases of the skin and in particular of diseases that are caused by dermatophytes, moulds and yeasts.

7. Use as claimed in claim 5 or 6 for the therapy or prophylactic treatment of psoriasis.

8. Use as claimed in claim 5 to remove hairs.

9. Agent for the prophylactic or therapeutic or cosmetic treatment of skin comprising chlorin E_6 and polyvinylpyrrolidone.

10. Agent as claimed in claim 9 comprising chlorin E_6 and polyvinylpyrrolidone in a weight ratio of 1 : (0.1 to 25).

11. Agent as claimed in claim 9 or 10, wherein the weight ratio of chlorin E_6 and polyvinylpyrrolidone is in the range of 1 : (1 to 25).

Abstract

The invention concerns an agent for the photodynamic diagnosis and therapy of oncological diseases based on chlorin E_6 compounds as well as new medical uses of chlorin E_6 compounds.

Figure 1: Accumulation of Fotolon in Pliss lymphosarcoma and healthy tissue

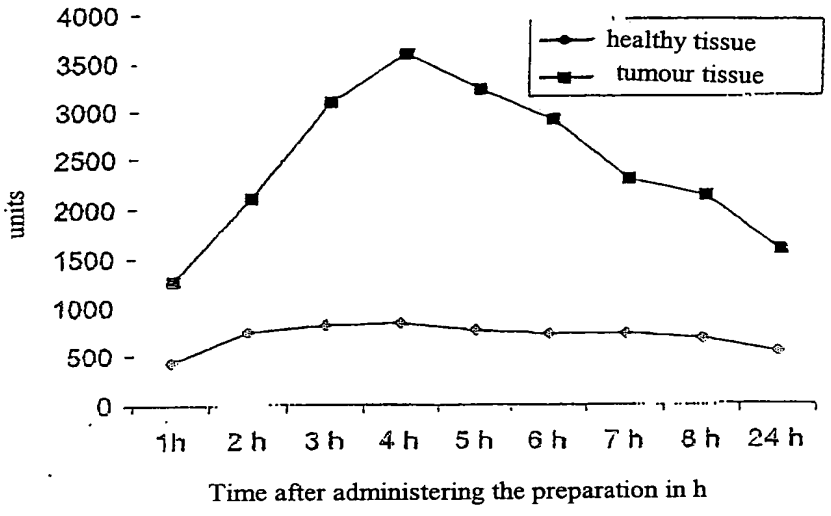

Figure 2: Accumulation of the agent according to the invention in Pliss lymphosarcoma and healthy tissue